Clonal Multiplication of Teak (*Tectona grandis*) by Using Moderately Hard Stem Cuttings: Effect of Genotypes (FG1 and FG11 Clones) and IBA Treatment

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Abstract

Teak (*Tectona grandis*) is one of the most important timber tree species in the Indian subcontinent and south-eastern countries. The study was performed to explore clonal propagation techniques by using moderately hard stem cuttings. The cuttings obtained from 20-year-old donors, namely FG1 and FG11, were treated with various concentrations of IBA, inserted into vermiculite, and cultured in a mist chamber at 32/26°C (day/night) and 85 ± 2% relative humidity. After 60 days, cuttings were removed from vermiculite and rooting assessment was made. FG1 clone was found superior in comparison to FG11 clone in terms of sprouting percentage, rooting percentage, roots per cutting and root length. With increase in concentration of IBA from 100 to 500 mg L^{-1}; there was an increase in rooting efficiency, as compared with the control. Results showed that moderately hard stem cuttings from FG1 clone, pretreated with IBA 500 mg L^{-1}, gave the best rooting traits. Therefore, these can be recommended for high quality planting stock material for clonal forestry programmes of teak.

Keywords

Clonal Variation; IBA; Moderately Hard Stem Cuttings; Vegetative Propagation; Rooting; Tectona Grandis

Introduction

Clonal propagation by cuttings is widely used to multiply elite trees obtained from the natural population to exploit the genetic variability. But rooting ability of cuttings varies for different types of cuttings and clones (Hartmann et al., 1997; Husen and Pal, 2003a, Husen, 2008). Several types of stem cuttings can be taken from the parent donors, which may be of hard, moderately hard, soft or herbaceous, depending on maturity of branch (Hartmann et al., 1997). Propagators usually select healthy, vigorous, well-matured, shoots with viable buds as the source of cuttings. Research has shown that the degree of maturity of stem cuttings plays an important role in rooting; and the cuttings obtained from young soft stem generally root more profusely (Hartmann et al., 1997; Husen 2012). In addition, loss of adventitious-root-regeneration potential in cuttings of forest tress with growing age of the donor plant is a common observation (Greenwood et al., 2001; Husen and Pal, 2006; Osterc et al., 2009; Husen, 2011; 2012; Husen and Khatoon, 2012). Adventitious rooting in cuttings has long been known to be affected directly by auxins, which can be either naturally occurring within the plant (endogenous) or applied to the plant (exogenous) during vegetative propagation. Usually IBA and NAA are recommended to promote adventitious roots in cuttings from shrubs (Husen and Mishra, 2001; Husen, 2002, 2003) or trees (Kaul, 2008; Husen, 2008a, 2012; Husen and Khatoon, 2012).

Teak (*Tectona grandis* Linn. f.) is a large deciduous tree and has gained a worldwide reputation on account of attractiveness and durability of its wood. Teak, a light-demanding species, does not tolerate shade or suppression at any stage of its life; and requires unimpeded overhead light for its proper development (Tewari 1992; Pandey and Brown 2000). Over most of its range, teak occurring in moist and dry deciduous forests below 1,000 m elevation is one of the several species constituting mixed forest stands and grows best in localities with annual rainfall of 1,250 - 3,750 mm, and a temperature range of 13 - 43°C, in addition, it prefers a deep, fertile, well-drained soil. Teak fails to grow in the soil with pH below 6.5; additionally its plantations have failed completely, when established on low-lying, poorly drained land with clay soils (Seth

and Yadav, 1959). Under favourable conditions, a tall clean cylindrical bole of teak is more than 30 m. Market demand has prompted establishment of teak plantations within and beyond its native countries. Teak has been successfully propagated vegetatively by grafting (Husen and Pal 2003a), hard stem cuttings (Husen and Pal 2000, 2007a) soft stem cuttings (Husen and Pal 2006, 2007b,c), split stem cuttings (Husen and Pal 2003b), and cuttings obtained from various age group of donor plants (Husen and Pal, 2006) and from etiolated stock plants (Husen, 2008a, 2011). However, currently no information is available on the effect of genotypes and the ability of moderately hard stem cuttings for rooting in teak. Thus, the objective of this study was to understand the individual as well as interactive effects of genotypes/clones and IBA treatments on adventitious root formation in moderately hard stem cuttings of teak.

Materials and Methods

Experimental site

The experiments were conducted at the Plant Physiology nursery of New Forest campus, Forest Research Institute (FRI), Dehra Dun, Uttarakhand, India, located in Doon Valley and surrounded by Western Lesser Himalayan ranges in the North and Shiwalik ranges in the South. This campus, on an area of 4.45 km^2, lies at an elevation of 640 m above mean sea level. It is situated on North Latitude 30° 20' 40" and East Longitude 77° 52' 12" on the northern limit of the Oriental region.

Donor plants

Twenty-year-old *Tectona grandis* clones (namely FG1 and FG11), chosen for this study, were raised by grafting and grown near Plant Physiology nursery, New Forest campus, FRI, Dehra Dun, India.

Stem cutting preparation

Moderately hard stem cuttings were collected from FG1 and FG11 clones. The partially matured cuttings (4-5 cm in length) were obtained in the summer month of July (Figure 1). The apical portion and leaves were removed and the upper part (pointing towards shoot apex) was sealed with paraffin wax. All cuttings were treated with 0.05% (w/v) bavistin for 30 min to avoid any fungal attacks during experimentation, and kept separately.

Treatments

As mentioned above, the cuttings were taken from two clones of teak, namely, FG1 and FG11. Indole-3-butyric acid (IBA) was used in five different concentrations i.e., 100, 200, 300, 400 and 500 mg L^{-1}. The cuttings were dipped in 1.0 percent solution of bavastin for 30 min and then treated with 100, 200, 300, 400 and 500 mg L^{-1} solution of IBA for 24 hrs by basal dip method. The control cuttings were treated similarly with distilled water only.

Rooting environment

After treatment, the cuttings were planted in plastic trays containing vermiculite (pH 7.0), which was presoaked for 24 hours in tap water before filling the trays. The culture trays were kept inside a mist chamber at 32/26°C (day/night) and 85 ± 2% RH; and the automatic day/night misting cycle was set to be 60/30s, with 1h gap between successive cycles.

Rooting assessment

Rooting and sprouting on cuttings (presence of at least one root/or shoot greater than 0.50cm in length) occurred within 2-4 weeks after planting. After 60 days, the cuttings were carefully removed from the rooting medium to record observation on sprouting percentage, rooting percentage, number of roots per cutting and average root length.

Statistical analysis

The completely randomized design (CRD) was used for experimentation with five replications (10 cuttings per replicate) and two factors, i.e., clones and IBA treatments. Data obtained on sprouting percentage and rooting percentage were transformed to arcsine $\sqrt{p}$ following the method of Anderson and Mclean (1974), while data on number of roots per cutting and average root length were used as such. Statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) computer software Version 6.1.3. In the analysis of variance (ANOVA) for parameters studied, mean values of each replication were estimated. For comparison of different means of different treatments; critical differences (CD) were calculated based on the Student's *t*-test at the $p \leq 0.05$ level. For analysis of variance (Table 1), the value for each replication was used based on all available cuttings and subjected to the model:

$$Y_{ij} = \mu + c_i + t_j + (ct)_{ij} + e_{ij}$$

Where, $_i$=1….2, $_j$= 1….6, Y_{ij} = response, μ = overall mean, c_i = effect due to i^{th} clones, t_j = effect due to j^{th} treatment of IBA, $(ct)_{ij}$ = interaction effect due to of i^{th} clones with j^{th} treatment of IBA, and e_{ij} = error term distributed N ~ $(0, \sigma^2)$.

Results and discussion

After 60 days, significant ($p \leq 0.01$) clonal variation was observed on sprouting and rooting percentage as well as the number of roots per cutting; while root length varied significantly at $p \leq 0.05$ level (Table 1).

The highest sprouting percentage (49.78%), rooting percentage (45.89), roots per cutting (2.17) and root length (3.28cm) were recorded in FG1 clone while these values were lowest for FG11 clone (Table 2). Overall, FG1 clone was found superior in terms of adventitious rooting in comparison to FG11 clone. This showed obvious variation between two clones with respect to rooting parameters. The FGI clone may be interesting for giving more uniform products and improving the forest plantation by using genetically better planting stock materials. In addition, FG1 also offers customer-tailored improved quality material as it has been known for being drought tolerant in comparison to FG11 clone (Husen 2010). Clonal variation in rooting efficiency has been reported previously for cuttings of several other plant species (Haines et al., 1992; Pounders and Foster 1992; Pal 1995; Husen and Pal 2003a; Husen 2004; Bakshi et al., 2005).

Further, the results showed that the moderately hard stem cuttings pretreated with various concentrations of IBA were significantly better ($p \leq 0.01$) for sprouting percentage, rooting percentage, roots per cutting and root length than the controls (Table 1). Increase in the exogenous IBA concentration (from 100 to 500 mg L^{-1}) enhanced the rooting efficiency (Table 3). The interaction effect of clones and IBA treatments was found significant ($p \leq 0.05$) for rooting percentage and number of roots per cutting (Table 1). The cuttings taken from FG1 clone and treated with 500 mg L^{-1} showed the highest rooting percentage and number of roots per cutting (Table 4), while the lowest values were recorded for untreated (0 ppm IBA) cuttings taken from FG11 clone. Enhanced adventitious root formation in cuttings by auxin treatments has been reported by many workers in teak as well as other plant species (Smart et al., 2003; Sorin et al., 2005; Bakshi et al., 2005; Husen and Pal 2006, Husen 2008b, 2012; Husen and Khatoon, 2012), and the present findings endorse those investigators.

In this study, sprouting and rooting started within 7-15 days after planting of cuttings under the mist chamber, but formation of shoots from the dormant buds of cuttings occurred much earlier. Formation of shoots earlier than roots in the case of teak may be due to presence of reserve carbohydrates in hard stem cuttings. The induction of more shoots by IBA application has also been reported in teak (Husen and Pal 2006, 2007c) and other plant species (Husen and Mishra 2001; Bakshi et al., 2005; Štefančič et al., 2005; Husen 2008b, 2012).

In conclusion, findings of this investigation suggest that clones/genotypes strongly affect rooting efficiency and shoot growth of teak cuttings. The FG1 have given the highest response. Therefore, moderately hard stem clone cuttings, treated with 500 mg L^{-1} IBA, stem cuttings of teak supplied with 500 mg L^{-1} IBA, can be recommended to achieve the best rooting response.

FIG. 1 ADVENTITIOUS ROOT REGENERATION IN MODERATELY HARD STEM CUTTING OF TECTONA GRANDIS

TABLE 1 ANOVA RESULTS ON THE EFFECT OF CLONES, IBA TREATMENTS AND THEIR COMBINATION ON ROOTING RESPONSE OF TECTONA GRANDIS

Source of variation	df	Mean sum of square			
		% sprouting	% rooting	Roots/cutting	Root length (cm)
Clones	1	4565.30**	3734.88**	11.83**	27.21*
IBA treatments	5	3323.89**	5445.44**	5.08**	5.44**
Clones x IBA treatments	5	110.36^{ns}	119.41*	0.85*	6.05^{ns}
Error	48	554.07	544.61	0.83	3.83
Critical difference at $p \leq 0.05$ level					
Clones		6.13	5.08	0.82	1.22
IBA treatments		4.40	6.82	0.20	0.62
Clones x IBA treatments		-	1.81	0.12	-

ns stand for insignificant; * and ** reflect significant at the $p \leq 0.05$ level and $p \leq 0.01$ respectively

TABLE 2 EFFECTS OF CLONE ON ROOTING RESPONSE IN MODERATELY HARD STEM CUTTINGS OF TECTONA GRANDIS

Teak clones	Rooting parameters			
	% sprouting	% rooting	Roots/cutting	Root length (cm)
FG 1	49.78 (40.76)	45.89 (44.41)	2.17	3.28
FG 11	34.89 (24.54)	26.82 (31.43)	1.75	2.67

Figures within parenthesis represent arc sin square root transformed values

TABLE 3 EFFECTS OF IBA TREATMENTS ON ROOTING RESPONSE IN MODERATELY HARD STEM CUTTINGS OF *TECTONA GRANDIS*

IBA treatments	Rooting parameters			
	% sprouting	% rooting	Roots/cutting	Root length (cm)
Control	12.58 (13.02)	7.33 (7.89)	0.68	0.97
100 mg L^{-1}	21.68 (20.48)	18.16 (17.48)	0.99	1.31
200 mg L^{-1}	44.84 (39.69)	41.99 (36.91)	1.56	2.68
300 mg L^{-1}	48.22 (43.18)	43.39 (38.26)	1.87	3.77
400 mg L^{-1}	58.53 (51.48)	50.79 (44.96)	2.48	3.88
500 mg L^{-1}	68.17 (59.69)	56.46 (50.35)	2.67	3.91

Figures within parenthesis represent arc sin square root transformed values

TABLE 4 INTERACTIVE EFFECT OF CLONES AND IBA TREATMENTS ON ROOTING RESPONSE IN MODERATELY HARD STEM CUTTINGS OF *TECTONA GRANDIS*

Parameters	Clones	IBA treatments					
		Control	100 mg L^{-1}	200 mg L^{-1}	300 mg L^{-1}	400 mg L^{-1}	500 mg L^{-1}
% sprouting	FG 1	14.66 (10.78)	28.04 (24.92)	56.35 (46.55)	56.44 (47.41)	68.63 (56.51)	74.57 (58.31)
	FG 11	10.50 (5.00)	15.33 (10.04)	33.33 (27.26)	40.00 (29.12)	48.44 (33.42)	61.78 (42.40)
% rooting	FG 1	10.66 (15.01)	26.67 (26.66)	53.32 (49.31)	54.12 (50.69)	64.26 (59.84)	66.26 (64.94)
	FG 11	4.00 (11.02)	9.66 (14.30)	30.66 (30.08)	32.66 (35.67)	37.33 (43.12)	46.66 (54.43)
Roots/cutting	FG 1	0.73	1.31	1.69	2.01	3.33	3.94
	FG 11	0.63	0.66	1.44	1.63	2.74	3.40
Root length (cm)	FG 1	1.08	1.72	2.03	3.38	5.24	6.24
	FG 11	0.86	0.90	2.51	3.33	4.17	4.30

Figures within parenthesis represent arc sin square root transformed values

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